Journal of Food Protection 88 (2025) 100511

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Real-time Detection of Foodborne Pathogens and Biofilm in the Food Processing Environment with Bactiscan, A Macro-scale Fluorescence Device

Callum Highmore [1,2,*], Kirsty Cooper [1,2], Joe Parker [1,2], Joshua Robinson [3], Roberto Castangia [3], Jeremy S. Webb [1,2]

[1] School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton SO17 1BJ Southampton, United Kingdom
[2] National Biofilms Innovation Centre (NBIC) and Institute for Life Sciences, University of Southampton, Southampton, United Kingdom
[3] EIT International, Biopharma House, Winchester, United Kingdom

ARTICLE INFO

Keywords:
Biofilm
Detection
Fluorescence
Food processing
Foodborne pathogen
Stress responses
VBNC

ABSTRACT

Food safety relies on rapid detection methods and rigorous sampling of the food processing environment and is challenged by recurrent biofilm contamination and by sublethally injured bacteria that can evade detection. Bactiscan is investigated as an alternative detection approach, a macro-scale and reagentless device that detects microbial contamination through activating the green fluorescence of glycoproteins in the bacterial cell wall. The detection capability of Bactiscan was tested on foodborne pathogens *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus*. Detection by Bactiscan was assessed using 3 independent observers viewing bacterial samples dried on stainless steel, using 3 biological repeats and 5 technical repeats. Detection by Bactiscan was possible to $1.20 * 10^6$ colony forming units (CFU), compared to $1.36 * 10^4$ CFU by ATP swab testing, where Bactiscan detection limits were defined by the concentration at which 50% of the samples were observed under illumination of the device. Heat-killed and chlorine-stressed *E. coli* and *S. enterica* caused a 2-log reduction in detection by ATP swab tests ($p \leq 0.05$), while detection by Bactiscan was unaffected ($p \geq 0.05$). Pathogen biofilms were detectable via Bactiscan with >80% accuracy at 4 days of growth; *E. coli* and *L. monocytogenes* biofilms were visible at 2 days of growth. *In situ* contamination studies determined that Bactiscan can detect microbial contamination on chicken, salmon, and yoghurt samples with stronger fluorescence than a competitor's UV torch. The presence of one of the pathogens on the food samples was confirmed by metagenome sequencing, determining that *S. aureus* was present in 7 samples out of 9 with a relative abundance of >0.5%. These data demonstrate that Bactiscan can effectively detect bacteria present in the food processing environment and can complement existing technologies to improve food industry cleaning practices and infection prevention.

Foodborne disease remains a profound challenge globally, where the World Health Organisation has estimated that there are 600 million cases of foodborne diseases annually and 420,000 deaths caused by 31 main pathogens (WHO, 2015). This burden is obscured by underdiagnosis and variation in disease surveillance systems, and further by unidentified causative agents of disease cases (Scallan et al., 2011). Furthermore, foodborne pathogens contribute to the estimated 1.3 billion tons of food wasted annually (Amani & Sarkodie, 2022). In the UK, *Listeria monocytogenes* was estimated to be responsible for only 126 cases in 2018, however, it claimed the highest mortality rate, with 21% of disease cases resulting in death and an estimated £230,700 associated cost per case (Daniel et al., 2020). *Escherichia coli* O157 has the second highest cost per case estimated at £8,400. Conversely, *Salmonella* spp. caused an estimated 31,601 disease cases in 2018 in the UK, with a total cost burden of £2.12 m, the second highest after *Campylobacter* spp. (£712.6 m) (Daniel et al., 2020). *Salmonella enterica* was also determined to cause the highest number of foodborne deaths and disability-adjusted life years globally (Pires & Devleesschauwer, 2021). While the transmission of these pathogens depends on undercooked or ready-to-eat foods, *Staphylococcus aureus* is responsible for the most prevalent type of foodborne intoxication globally, via the secretion and persistence of staphylococcal enterotoxins in food following processing treatments (Fetsch & Johler, 2018). These toxins are difficult to detect, where the food matrix inhibits the efficacy of commonly used immunoassays. Due to the low infectious doses (Schmid-Hempel & Frank, 2007), severity of infection (Daniel et al.,

* Corresponding author at: School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, SO17 1BJ Southampton, United Kingdom.
E-mail address: C.J.Highmore@soton.ac.uk (C. Highmore).

https://doi.org/10.1016/j.jfp.2025.100511
Received 13 May 2024; Accepted 15 April 2025
Available online 21 April 2025

2020), and durability of enterotoxins (Fetsch & Johler, 2018), the prevention of foodborne disease outbreak relies heavily on the detection of contaminants within the food processing environment.

Contamination of food in factories can originate from biofilms established on produce harvesting equipment or factory surfaces and equipment (Manville et al., 2023). One survey estimated that this transfer is the cause of 60% of foodborne disease cases (Bridier et al., 2015; Saadi et al., 1998). The transfer of pathogen biofilm from food processing environment to food itself was demonstrated by Wang et al, where the transfer of *S. enterica* cells from a stainless steel surface to beef tissue correlated to the biofilm formation ability of the bacterial strains tested (Wang et al., 2022). The biofilm phenotype is particularly significant for the survival of foodborne pathogens, as it provides physical and chemical protection from standard decontamination procedures (Bridier et al., 2011; Simões et al., 2003), and complex microbial communities can augment the growth and persistence of foodborne pathogens (Bai et al., 2022). Furthermore, the adaptive heterogeneity of biofilms permits regrowth and recurrent contamination following cleaning practices (Bridier et al., 2015). Habimana et al determined that *L. monocytogenes* was incorporated into established *Lactococcus lactis* biofilms depending on rugosity and relative quantity of extracellular polymeric substance (EPS) components (Habimana et al., 2009). Biofilms can also contribute to the severity of foodborne disease cases, via facilitation of horizontal gene transfer for antimicrobial resistance genes. This was demonstrated between *Pseudomonas putida* and *E. coli* (Van Meervenne et al., 2014), where a plasmid conferring multidrug resistance was transferred between the two species, with an incidence between 2 and 10% of the recipient population. An upregulation of virulence factors was observed in biofilm-derived *L. monocytogenes* during adaptation to the intestinal environment (Bai et al., 2021), further demonstrating the increased risks to food safety posed by biofilm formation.

Despite the relative size and significance of biofilm contamination in the food processing environment, difficulties in effective sampling and sanitization impede the microbial safety of food products. In industry, nonspecific detection of bacterial contamination relies on ATP swab testing (Bottari et al., 2015; Luo et al., 2009), where specificity is primarily determined by traditional culture techniques. One study confirmed that ATP testing correlated to culture plate counts of *L. monocytogenes* in deli surfaces (Hammons et al., 2015). It was found that a relative 1-log increase in ATP detection corresponded to a fourfold increase in the probability of *L. monocytogenes* detection by culture plate, although did not reflect the quantity of *L. monocytogenes* contamination. While ATP swab tests are rapid and sensitive, disadvantages include the small sample area and the nonspecific detection of ATP from food residues that may lead to false-positive results (Bottari et al., 2015). ATP tests are also not optimized for the detection of stressed or viable but nonculturable (VBNC) bacteria, due to their reduction in metabolic activity (Robben et al., 2019).

Traditional culture techniques are considered the 'gold standard' (Foddai & Grant, 2020), and the microbial safety of food relies heavily on the use of culture media. However, these techniques are slow and inefficient at containing contamination events, so alternative detection methods including ATP swab tests and molecular detection techniques are increasingly used. PCR-based methods such as qPCR are sensitive and specific, and can be modified using propidium monoazide (PMA) to selectively detect viable foodborne pathogens including *Salmonella* spp. and *E. coli* O157:H7 in complex food samples (Li & Chen, 2012, 2013; Zeng et al., 2016). The PMA technique has been applied to a rapid alternative DNA amplification technology, loop-mediated isothermal amplification (LAMP), and was used by Han et al to detect VBNC *S. enterica* and *E. coli* O157:H7 within 30 min in spiked spinach at 1.05 * 10^5 and 5.13 * 10^4 CFU/g, respectively (Han et al., 2020). The increasing prevalence of whole genome sequencing has led to new tools for pathogen detection in food samples, for example, Merda et al. have constructed a bioinformatics pipeline to facilitate the rapid identification of *S. aureus* strains following foodborne disease outbreaks (Merda et al., 2020).

Hyperspectral imaging (HSI) is an emerging approach in microbial detection that encompasses a broad range of spatially resolved light-based technologies to classify the presence of bacteria, offering the advantage and macro-scale detection. Typically, HSI involves acquisition of an image series of a sample taken at multiple wavelengths of light, and combining them to generate a "hyperspectral datacube" that displays spectroscopic variation which influences microbial detection (He & Sun, 2015). HSI data are increasingly being analyzed using machine learning tools to improve classification accuracy of contamination identification on foods (Gowen et al., 2015; Soni et al., 2022). Jun et al measured the fluorescence of *E. coli* and *Salmonella* biofilms on a range of surfaces using HSI in the UV-A spectrum, suggesting that a fluorescence-based detection method could be valuable in pathogen detection in the food industry (Jun et al., 2010).

Pathogen detection methods currently adopted by the food industry require either significant time, specialist expertise, or the continued use of consumables. As an alternative detection methodology, this study assesses the use of Bactiscan, proprietary technology that detects bacterial contamination on the macro-scale using four UV lamps that excite bacterial components to produce blue-green fluorescence. This study aims to measure the sensitivity of the instantaneous fluorescence-based detection of bacterial contaminants by Bactiscan, and compare its ability to detect prominent foodborne pathogens with other detection technologies currently used in the food processing sector. To achieve this, the detection capability of Bactiscan was assessed using bacterial biofilms, and living, dead, and stressed cell populations, as examples of different bacterial states that might be present in a food processing environment. Contamination events were simulated on real food samples to determine the efficacy of Bactiscan in detecting bacterial contamination *in situ*.

Materials and methods

Bacterial strains and culture methods. Foodborne pathogens used were *E. coli* NCTC 12900, *L. monocytogenes* Scott A, *S. enterica* Typhimurium ATCC 12023, and *S. aureus* UAMS-1. These bacterial species were chosen for this study as they are prominent foodborne pathogens that represent Gram-positive and Gram-negative species with bacilliform and coccoid morphologies. Planktonic bacterial cultures were grown overnight in brain-heart infusion broth at 37 °C, where *S. aureus* was grown with aeration. Bacteria were grown in brain-heart infusion agar overnight at 37 °C for enumeration. Biofilms of each bacterial species were grown in 20% strength brain-heart infusion broth with an inoculum concentration of 10^5 CFU/mL, 4 mL inoculum was placed into a polystyrene 6-well plate containing a glass microscope coverslip (22 mm × 22 mm). Steel was considered as a biofilm substrate due to its presence in the food processing environment, but glass was chosen as it is a substrate typical to biofilm research, to ensure reproducibility. Biofilms were incubated at 37 °C with gentle agitation at 50 rpm for 10 days with daily growth media replacement. Bacteria were stored at −80 °C in brain-heart infusion broth with 25% glycerol (v/v).

Experimental design. The Bactiscan device was supplied by EIT International, a standard model manufactured in 2022, serial number 21086. The Bactiscan was calibrated in June 2023; control voltages within the unit were checked against a standard, and excitation frequency and resulting spectra of the LEDs were validated prior to the commencement of experiments. The device is a handheld torch that emits four wavelengths of light in the UV-A spectrum, the light reacts with the bacterial target to produce green fluorescence. It was theorized that the excitation of bacterial S-layer causes the fluorescence, as it is a crystalline glycoprotein coat that spans the surface of many Gram-positive and Gram-negative bacteria and S-layer proteins are

one of the most abundant proteins in the bacterial cell (Fagan & Fairweather, 2014). *E. coli* and *S. enterica* do not possess S-layers, but were illuminated under Bactiscan (Fig. S1), so the presence of S-layers cannot explain the extent of the source of bacterial fluorescence under Bactiscan. Other materials such as dust and food residues produce white-blue fluorescence.

For experiments determining the detection limit of Bactiscan, planktonic bacterial cultures were grown and washed in ddH_2O by centrifugation and then prepared to a range of concentrations by serial dilution in ddH_2O. Concentration of planktonic cultures was confirmed by colony counts on brain–heart infusion agar. Suspensions of bacteria in ddH_2O were dried at 45 °C onto 75 mm × 25 mm steel surfaces (type 304L grade 2B) for observation. Samples were dried using a heat block and stored in sterile Petri dishes during the drying process. Biofilm samples were prepared for detection by removing all culture media before observation and washed once in phosphate-buffered saline. Each volume and concentration of planktonic bacteria, and each biofilm sample, were sampled with three biological repeats, with five technical repeats for each biological repeat. Experiments were conducted in ambient light and in darkness. The experimental setup is shown in Figure 1. The light source of the Bactiscan device was held 20 cm from the sample under observation, and technical repeats of bacterial samples were illuminated together on stainless steel coupons. The 20 cm distance was used as it is the average distance end users position the device from the target surface, as a balance between device focus and diffraction properties, and typical factory light levels. Biological repeats were illuminated separately.

Observation and defining the detection limit. Observation of each sample was carried out by three independent observers, who recorded their observation of each sample as a binary 'yes' or 'no'. Observers were scientists that were otherwise unconnected to the present study. These observers had no prior experience using the Bactiscan device to emulate potential future users of the device in industry. Percentage of observation was calculated by comparing the total number of positive observations against the total number of samples tested, pooling data from all observers, biological, and technical repeats. The detection limit for Bactiscan was defined as the concentration at which the sample was observed with a 50% success rate across the three observers. The LOD50 was used to define the limit of detection of Bactiscan as it marks the microbial concentration at which an area of contamination is equally likely to be detected or undetected, in line with the limit of detection of a culture plate count. Alternative detection limits were proposed such as the 'subjective detection limit', the concentration at which $<100\%$ were observed, and an 'objective detection limit', the concentration at which no samples were observed as positive by any of the observers. These alternative detection limits were considered unsuitable due to the high subjectivity associated with measurement by human eye. Despite the inherent error that comes with this approach, measuring the efficacy of Bactiscan as a microbial detection method via human observation is necessary because that is how the technology is used in industrial settings.

Detection of stressed bacteria. Bacteria were prepared in planktonic cultures to concentrations of 5.0 * 10^8 CFU/mL, verified by culture plate counts, and either heat-killed by incubating at 70 °C in 2 mL Eppendorf tubes using a water bath for 30 mins, or chlorine stressed by exposure to 50 ppm chlorine in liquid culture for 3 min as established in previous work (Highmore et al., 2018). Briefly, 1 mL of aliquots of planktonic bacterial culture at 5.0 * 10^8 CFU/mL was inoculated into

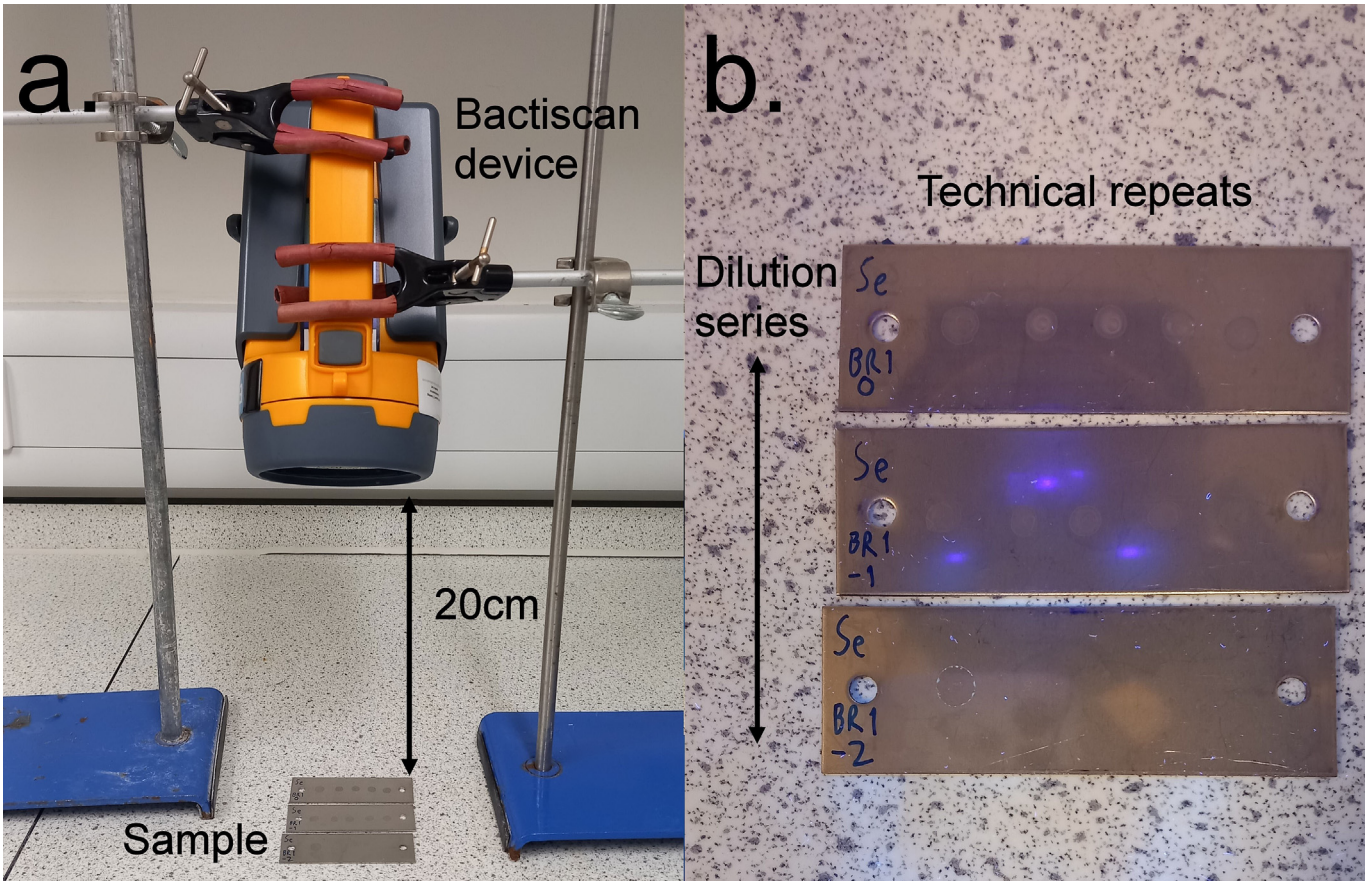


Figure 1. A photograph of the experimental setup for detection of microbial contamination by Bactiscan. (a) The Bactiscan light source is held 20 cm from the bacterial sample, dried onto steel coupons. (b) There are 5 technical repeats present on each steel coupon, and three dilution factors can be illuminated under Bactiscan at once. Biological repeats were examined separately.

50 ppm chlorine in ddH_2O (50 mL total volume) for 3 min. The chlorinated water was removed using vacuum filtration, and the sample was retained on a 0.22-μm-pore-size mixed cellulose ester membrane (Millipore, USA). Bacterial samples were removed from the membrane by vortexing for 30 s and washed in ddH_2O following treatment, then dried onto a steel surface for observation as described above. The inoculum concentration used in this study was higher than would be found in the food processing environment so that the extent of the effect of stressed bacterial states could be accurately measured.

ATP testing. ATP swab testing was performed using a Hygiena SystemSURE Plus Luminometer (Hygiena, California, USA) and UltraSnap Surface ATP test swabs (Hygiena, California, USA) according to the manufacturers' instructions. Swabs were applied to bacterial samples prepared and dried onto steel as described above. Test swabs were shaken for 5 s before insertion into the luminometer. ATP activity was measured and recorded as relative light units (RLU), to ensure applicability of the data to the food processing environment. Three replicate swabs were taken for each bacterial sample. ATP testing was carried out in conjunction with Bactiscan analysis to compare the detection of living, dead, and stressed cells using both methods.

Microscopy analysis. Biofilms were visualized by confocal laser scanning microscopy using a Leica SP8 microscope at 24, 48, and 72 h time points. Samples were stained with 1% SYTO 9 and propidium iodide and incubated in darkness for 20 mins before observation under 488 nm and 514 nm excitation. Representative images for each biofilm species and age were generated via acquiring z-stacks with 0.33 μm step sizes, and were processed using Imaris 9.9, Oxford Instruments – Andor, https://imaris.oxinst.com. Three representative images were taken per biofilm per time point.

Food contamination and metagenome sequencing. Ten grams each of chicken thighs (Tesco, UK), salmon fillets (Tesco, UK), and yoghurt (Yeo Valley, UK) representing meat, fish, and dairy, respectively, were smeared 10 cm across surfaces commonly found in the food processing environment, Teflon, steel, and polyurethane. They were incubated at room temperature for 5 days to permit the growth of natural microflora present on the foods, and photographed daily under illumination by Bactiscan and a competitor UV torch in ambient light and in darkness. On the 5th day, a 3×5 cm^2 area of each of the contaminated surfaces was swabbed and subjected to DNA extraction using a DNeasy Powerbiofilm Kit (Qiagen). Samples were eluted in 100 μL elution buffer and quantified using a BioDrop Duo+ (Table S1) and then submitted to Novogene (Cambridge, UK) for sequencing. Samples were subjected to Illumina amplicon metagenomic sequencing with 250 paired−end reads and a sequencing depth of 30,000 tags per sample. Taxonomic classification of reads was performed using Kraken2 (Wood et al., 2019) following sequencing, all reads with Q-score above Q30 were retained for downstream analyses, querying against the Standard database (accessed 07th June 2022) with default parameters. Any taxa with less than 0.5% representation were filtered from the analysis.

Statistical analyses. Statistical analyses were performed with Graphpad Prism version 9.5.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com. One-way ANOVA with Fisher's least significant differences test was used to compare data collected from different observers in the detection limit experiments. Two-way ANOVA with Tukey's multiple comparisons test was used to measure differences in ATP test swab conditions ($p \leq 0.05$), and lin-

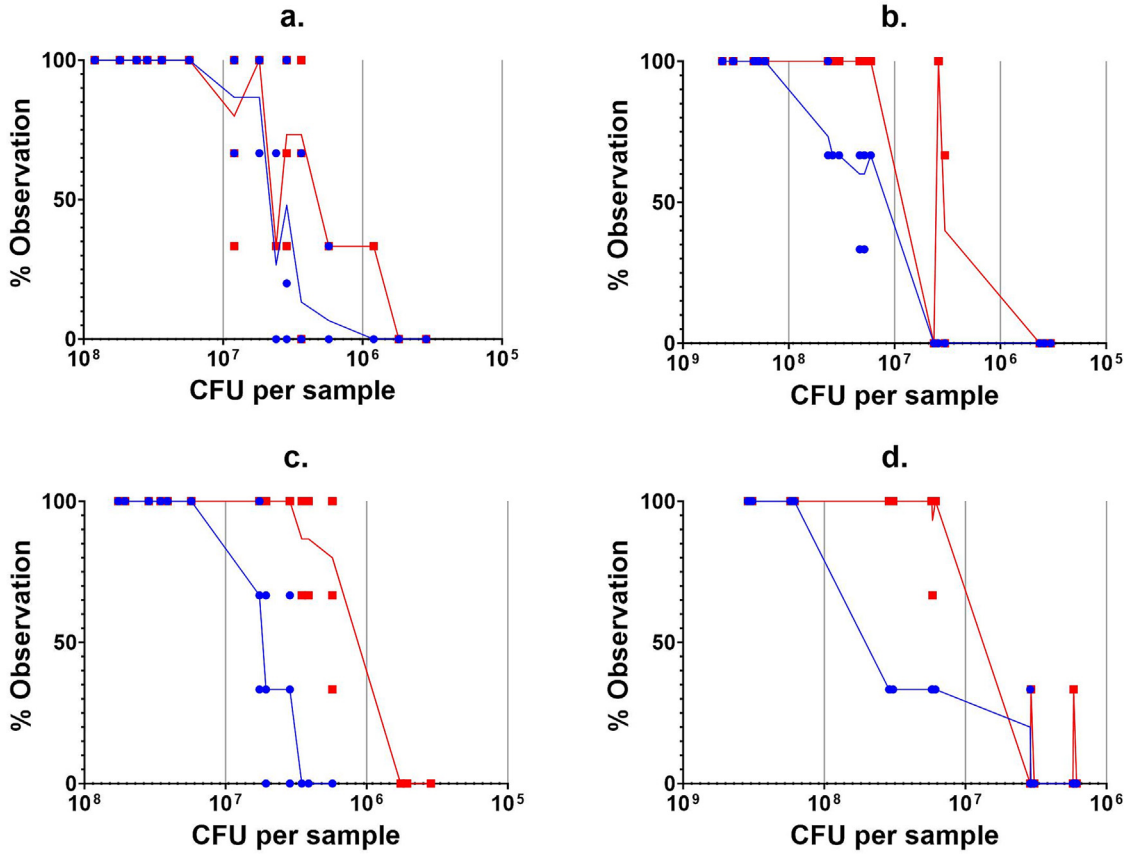


Figure 2. Percentage observation of foodborne pathogens (a) *E. coli*, (b) *L. monocytogenes*, (c) *S. enterica*, and (d) *S. aureus* under Bactiscan illumination in daylight (blue) and in darkness (red). Connecting line indicates mean observation percentage, individual replicates shown as points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ear regression was used to calculate bacterial CFU numbers from RLU measurements.

Results

The bacterial detection limit of Bactiscan. Foodborne pathogens *E. coli*, *L. monocytogenes*, *S. enterica*, and *S. aureus* were dried onto steel surfaces at a range of concentrations and volumes in ddH_2O (Fig. 2). Each condition was observed by 3 independent observers to establish the LOD50 detection limit for each pathogen (Fig. 2, Table 1). The subjectivity of human observation caused significant variation in the data when defining detection limits, e.g. observers could detect 3.85 * 10^6 CFU *L. monocytogenes* but did not detect a higher concentration of 4.25 * 10^6 CFU (Fig. 2b). The data show a range of concentrations of microbial contamination at which detection by the observers is highly variable. Despite this variability, this methodology was necessary to ensure the Bactiscan device was assessed in the same manner that it is used in food processing environments. A statistical difference was found between data collected by each observer for Bactiscan detection of three of the four pathogens when experiments were conducted in daylight; there was no difference between observers' detection of *S. enterica*. No difference was measured between different observers for experiments conducted in darkness. *Salmonella enterica* in the darkness had the lowest LOD50, at 1.20 * 10^6 CFU, and *S. aureus* had the highest LOD50 in ambient light, at 5.37 * 10^7 CFU (Table 1). No inherent difference in brightness of fluorescence was observed between the different bacterial species (Fig. S1), although this may be a valuable area of future study.

Detection of biofilm by Bactiscan. Biofilms of foodborne pathogens were grown and observed each day under illumination by Bactiscan. Biofilms of all species could be reliably detected within 4 days of growth, where >80% of positive samples were observed (Fig. 3). Prominent foodborne pathogens *E. coli* and *L. monocytogenes* could be consistently detected from day 2, *S. aureus* by day 3 and *S. enterica* by day 4. To determine the factors that influence Bactiscan illumination of biofilms, confocal microscopy images were taken daily for the first 3 days of biofilm development, assessing the effect of cell viability and biovolume on detection by Bactiscan (Fig. 4).

Observation of stress states of foodborne pathogens. To determine whether microbial detection by Bactiscan is impeded by different microbial physiological states, heat-killed and chlorine-stressed bacterial populations were prepared to concentrations of 5.0 * 10^8 CFU/mL. Foodborne pathogens were either left untreated, heat-killed at 70 °C in a water bath, or chlorine-stressed with 50 ppm chlorine for 3 min, before observation under Bactiscan illumination. The pathogens were air-dried on steel from suspension in ddH_2O, causing a ring effect when dried. In darkness, heat-killed bacteria were observed uniformly as well as untreated live bacteria, however, chlorine-stressed *S. aureus* fluoresced only faintly (Fig. 5). *E. coli* and *L. monocytogenes* displayed the same fluorescence pattern as *S. enterica.*

Table 1
The concentration at which 50% of each foodborne pathogen was observed (LOD50), determined by linear regression

	Detection limit (CFU)	
Bacterial species	Light	Dark
E. coli	3.33 * 10^6	1.94 * 10^6
L. monocytogenes	1.65 * 10^7	9.32 * 10^6
S. enterica	5.46 * 10^6	1.20 * 10^6
S. aureus	5.37 * 10^7	9.02 * 10^6

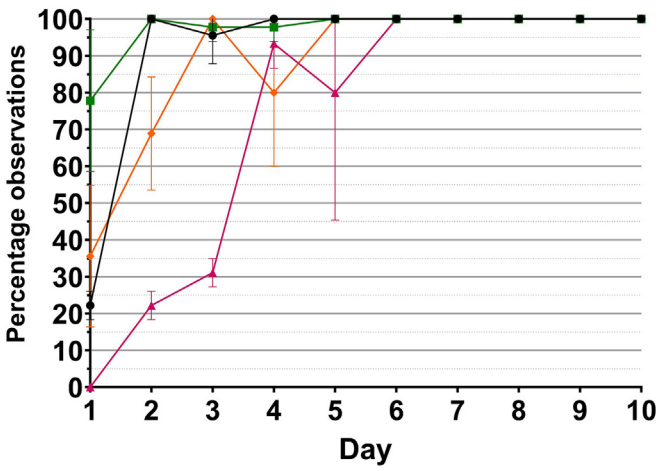


Figure 3. Detection of *E. coli* (black), *L. monocytogenes* (green), *S. enterica* (pink), and *S. aureus* (orange) biofilms by Bactiscan grown at optimal conditions (at 37 °C, viewed in darkness). Error bars indicate standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

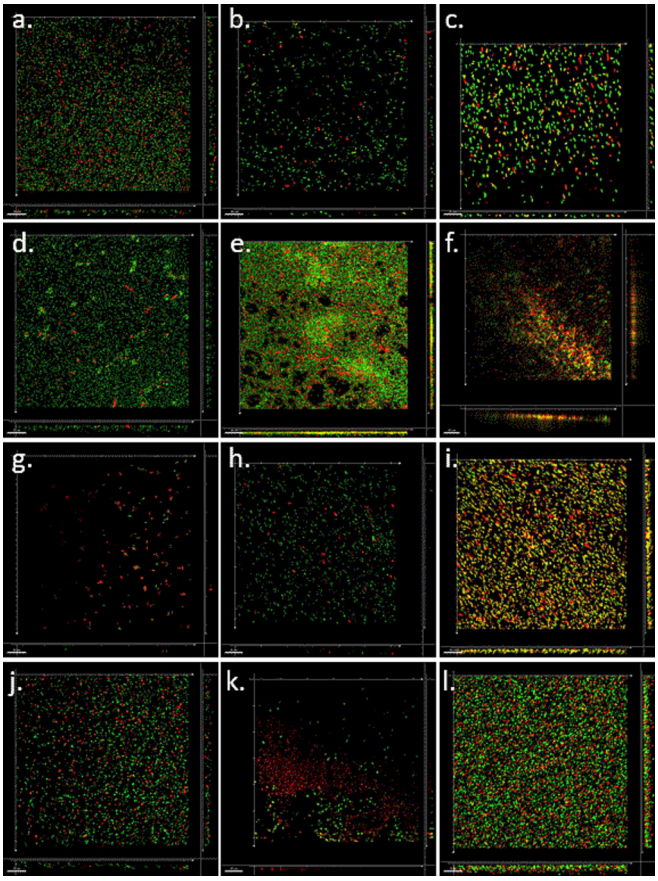
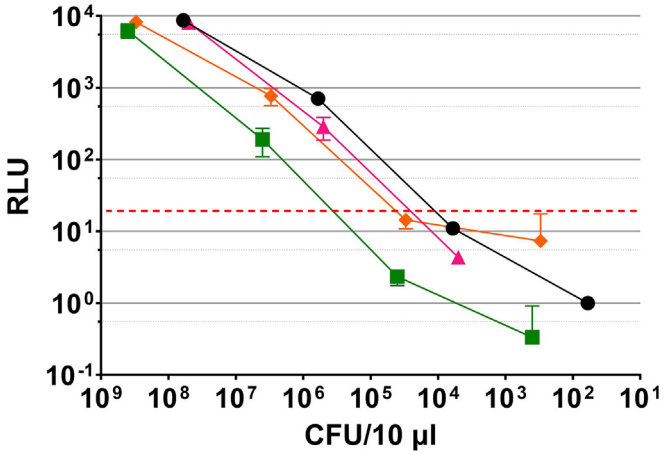


Figure 4. Representative confocal microscopy images of (a–c) *E. coli*, (d–f) *L. monocytogenes*, (g–i) *S. enterica*, and (j–l) *S. aureus* biofilm after (a, d, g, j) one, (b, e, h, k) two, and (e, f, i, l) three days of growth, stained with SYTO 9 (green) and propidium iodide (red) indicating live and dead cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Comparison with ATP swab tests. To compare Bactiscan to current methods of contaminant detection in food processing plants, surface ATP testing swabs were assessed for a comparative detection limit (Fig. 6). Linear regression was used to determine the RLU that the ATP test swabs would emit at the LOD50 of Bactiscan and found that the RLU ranged from 246 to 1120 for the bacterial species tested. The detection limit for ATP test swabs was >1-log lower than that of Bactiscan when applied to living bacterial cell populations. Heat-killed and chlorine-stressed bacteria were detected by ATP test swabs with reduced sensitivity, equivalent to a 2-log reduction in the detection of *E. coli* and *S. enterica*. Detection of *L. monocytogenes* and *S. aureus* had a less prominent reduction, of 62% and 89%, respectively (Fig. 7). Heat and chlorine treatments did not cause a reduction in detection by Bactiscan. Detection of each pathogen species subjected to different stress conditions by ATP swab tests was not statistically different ($p \geq 0.05$).

Detection of contamination of food samples. Meat (chicken), fish (salmon), and dairy (yoghurt) were smeared across three surfaces common to the food processing environment (Teflon, steel, and polyurethane) and observed daily under illumination by Bactiscan and a competitor UV torch for 5 days (Figs. 8–9). The Bactiscan device caused the contaminated areas to fluoresce more brightly than the UV competitor torch, with a strong turquoise color. The UV torch was less consistent with color, where dairy contamination was illuminated white, and meat and fish were a weaker blue. The UV torch illuminated the surface to the detriment of contamination detection, particularly on steel, where reflection of the UV light obscured the contamination sites. Bactiscan was also more effective at illuminating small areas of contamination and distinguishing between food matter and microbial communities.

After 5 days of incubation, the contaminated areas were swabbed and subjected amplicon sequencing to determine the microbial makeup of contamination observed under Bactiscan illumination (Fig. 10). *Carnobacterium maltaromaticum* was present in all samples and was the primary bacterial component of each meat sample and one fish sample. Each of the dairy and fish samples had a different bacterial species with the greatest relative abundance. Dairy samples of this study contained *Streptococcus thermophilus*, a suspected probiotic species frequently utilized in yoghurt production (Martinović et al., 2020), and the dairy sample incubated on polyurethane contained 66.23% *Corynebacterium resistens*. Seven of the nine samples contained *S. aureus* with a relative abundance of >0.5%, and fish incubated on Teflon had a *S. aureus* relative abundance of 85.95%. *Salmonella* was present in the fish sample incubated on polyurethane, but at a relative abundance of 0.54%, it is unlikely that it significantly contributed to



Figrue 6. Relative light units (RLU) indicating detection of bacterial species *E. coli* (black), *L. monocytogenes* (green), *S. enterica* (pink), and *S. aureus* (orange) by ATP test swabs. Red line indicates the RLU reading that denotes a failed test, according to the luminometer used. Error bars indicate standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

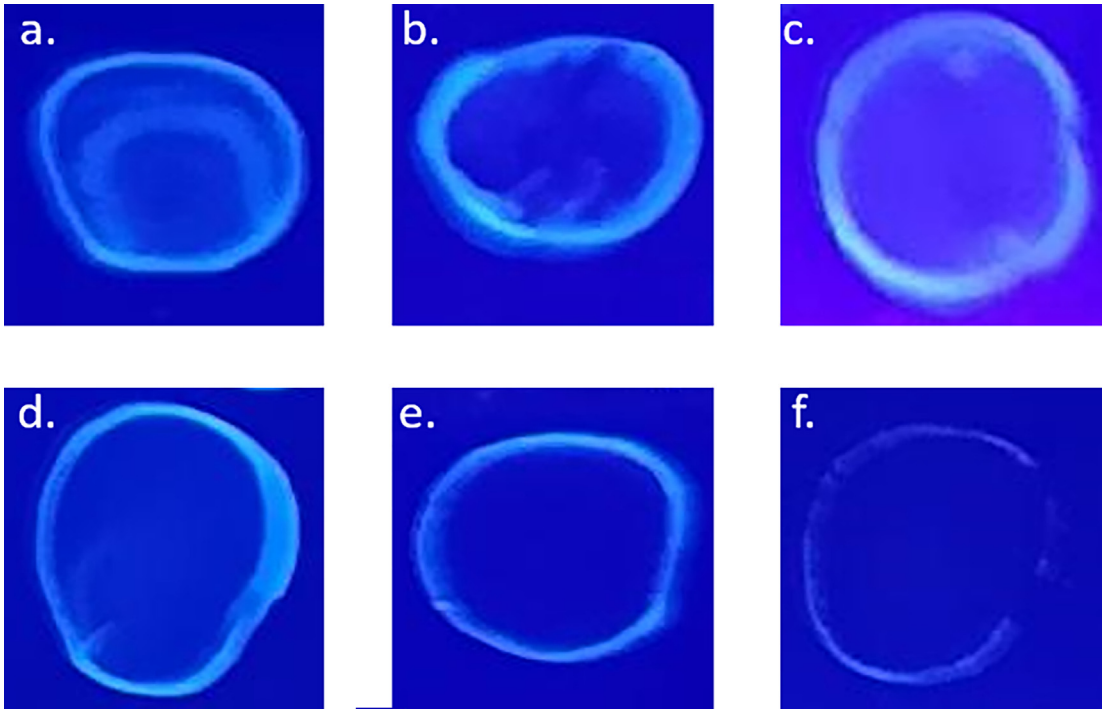


Figure 5. Representative images of dried (a-c) *S. enterica* and (d-f) *S. aureus* inocula treated with different stressors: untreated (a, d), heat-killed (b, e), and chlorine stressed (c, f) and illuminated under Bactiscan.

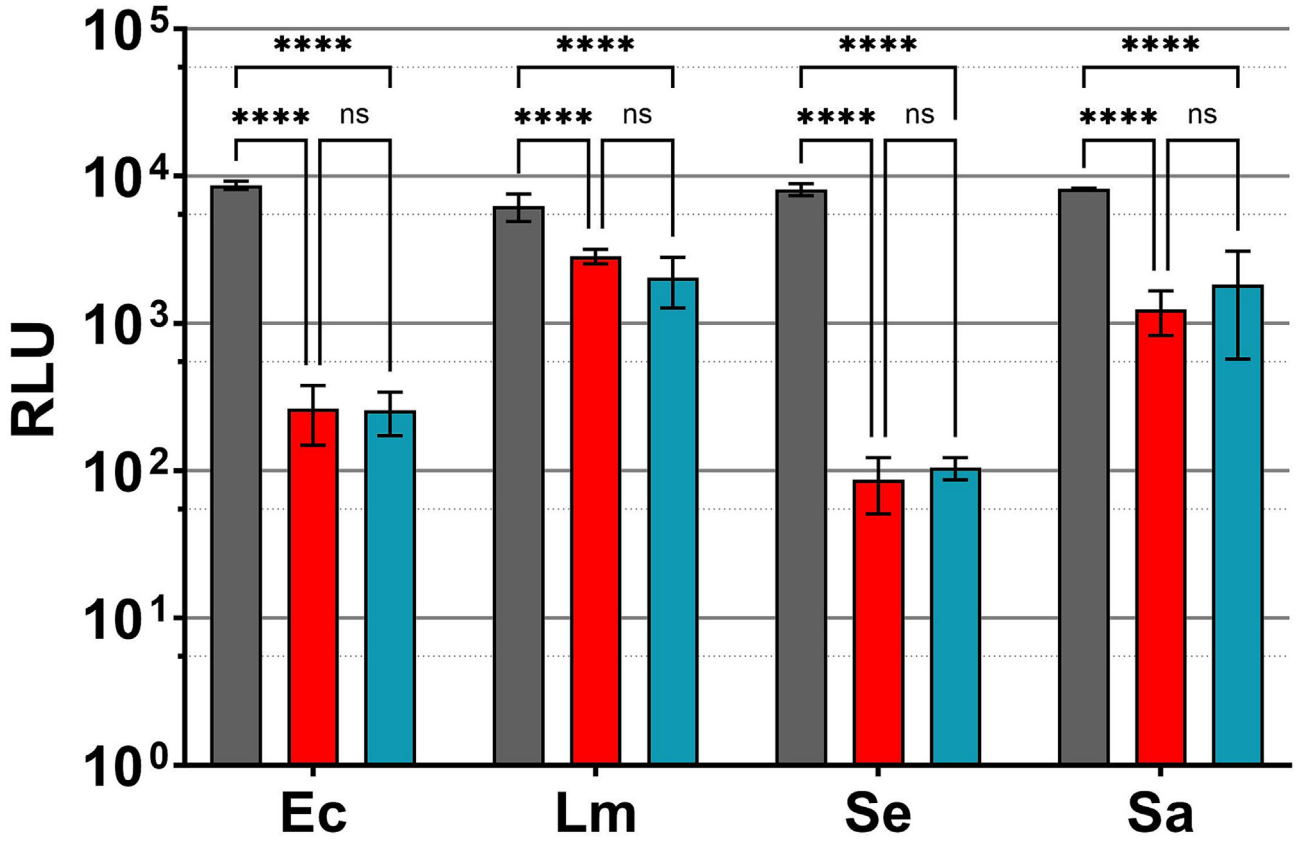


Figure 7. Relative light units (RLU) indicating detection of living bacterial species (grey), heat – killed bacteria (red), and chlorine-stressed bacteria (teal) by ATP test swabs. Significance was calculated by 2-way ANOVA with Tukey's multiple comparisons test ($p \leq 0.05$). Error bars indicate standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

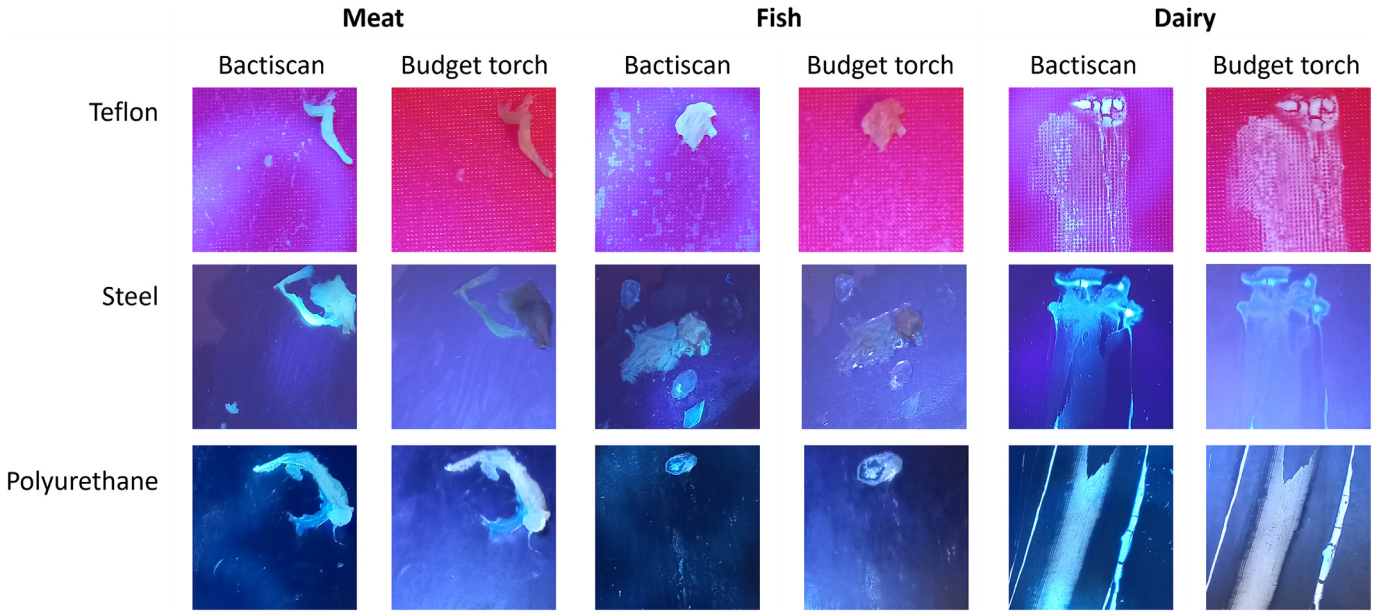


Figure 8. A comparison of macro-scale detection methods (Bactiscan and a competitor budget UV torch) for contamination of food products incubated for five days (meat, fish dairy), using representative images taken in ambient light.

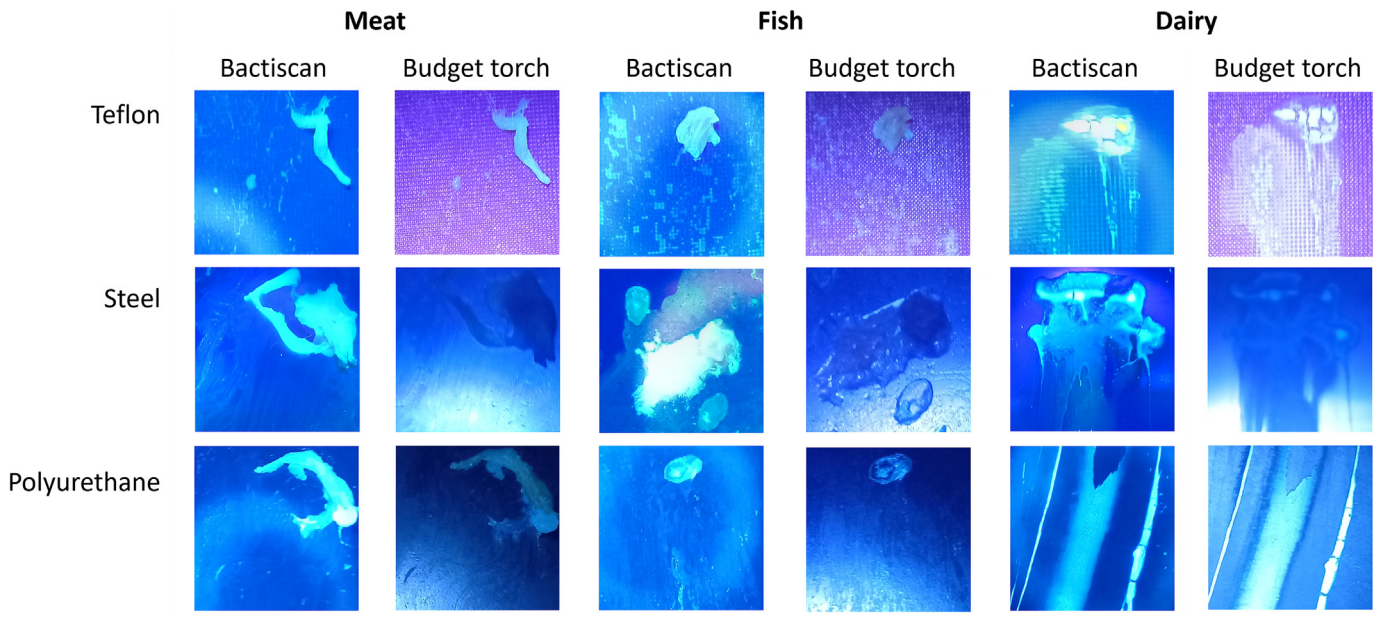


Figure 9. A comparison of macro-scale detection methods (Bactiscan and a competitor budget UV torch) for contamination of food products incubated for five days (meat, fish dairy), using representative images taken in darkness.

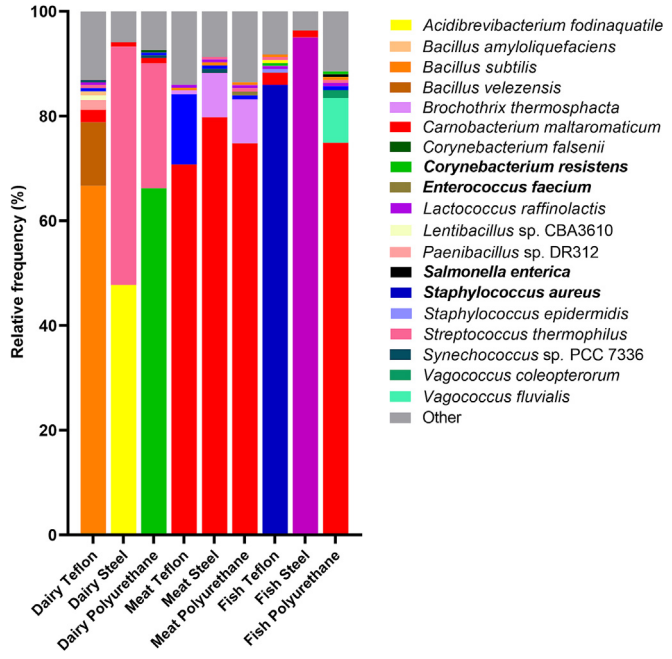


Figure 10. Bacterial community analysis at the species level from food samples incubated on different surfaces at room temperature for 5 days. Each species with a relative abundance of above 0.5% is specified. Human pathogens are labeled in bold.

contamination detection via Bactiscan illumination in this sample (Fig. 10).

Discussion

The Bactiscan device facilitates observation of 5.46 * 10^6 CFU *S. enterica* in a 5 μL contamination inoculum (Table 1), although observation was possible at 7.0 * 10^5 CFU *S. enterica* in 2 μL contamination spots. This study aimed to establish detection limits for prominent foodborne pathogens by Bactiscan. However, the nonlinear reduction of observation with bacterial concentration highlights the extent of subjectivity when using detection tools that rely on human judgment (Fig. 2). For example, *S. aureus* was not observed by two observers in ambient light at a concentration of 3.47 * 10^7/10 μL, but was observed by a third observer, causing a prominent disparity between detection of *S. aureus* in light and darkness. Perhaps when used by experienced operators in industry, the variability in microbial contamination detection by Bactiscan will decrease over time. The limit of detection by ATP testing for the pathogens tested is between 1.36 * 10^4 CFU (*E. coli*) and 4.11 * 10^5 CFU (*L. monocytogenes*) (Fig. 6). This is more sensitive than observation by Bactiscan; however, it must be noted that the experimental procedures implemented, using metabolically active planktonic populations of bacteria in small, defined areas, are particularly suited to the ATP testing that do not translate to the food processing environment where areas are swabbed randomly. Previous studies using HSI devices (Beck et al., 2015; Jun et al., 2010; Wiederoder et al., 2013) determined that macro-scale fluorescence imaging is most effectively employed in food processing settings by identifying areas of contamination in real-time for further cleaning, and verification of sterility should then be achieved using ATP swab tests.

The ubiquitous and persistent nature of biofilms necessitates effective detection techniques in the food processing environment (Saadi et al., 1998). Under optimal conditions (grown at 37 °C, viewed in darkness), biofilms of three of the four tested species were reliably detectable by Bactiscan from 2 days of growth (Fig. 3). To account for the heterogeneity of the biofilm phenotype, representative microscopy images of each biofilm were taken for the first 3 days to compare visibility by Bactiscan to biofilm viability and morphology (Fig. 4). While cell viability, biovolume, and biofilm architecture were measured to identify correlation with detection by Bactiscan, these did not adequately explain differences in detection, such as the relatively low detection of *S. enterica* biofilm by day 3. *Salmonella enterica* biofilms have previously been grown on glass and have required incubation for >14 days or supplementation with 3% bile to increase biomass (Prouty et al., 2002), which may partially explain its relatively low detection at 3 days of growth. These data highlight that compre-

hensive analysis on the fluorescence of different microbial species is an important subject of future work.

Heat-killed cells are as visible as living cells under illumination by Bactiscan (Fig. 5), so it is likely that biofilm detection is less influenced by cell viability than it is by total cell numbers. *Staphylococcus aureus* cells stressed with chlorine exposure were less visible than heat-killed cells. This could be due to the mechanism of action of reactive chlorine species in the bacterial cell, which leads to oxidization and unfolding of proteins in the cell wall (Fagan & Fairweather, 2014; Gray et al., 2013), which are critical to detection by Bactiscan. Damage to the cell wall is likely to be less extreme in heat-killed bacteria (Russell, 2003) leading to the difference in visibility. In mature biofilms, cell death commonly occurs due to selection pressures such as nutrient deprivation, which will not necessarily destroy the integrity of the S-layer and other protein sources of fluorescence. This may support the use of the device in the food processing environment, where dead and stressed cells comprise an integral part of the biofilm ecosystem.

Due to the binary nature of bacterial detection by Bactiscan and despite the reduced fluorescence of chlorine-stressed *S. aureus*, the detection of foodborne pathogens was not impaired by chlorine stress. However, the effect of chlorine stress on the Bactiscan detection limit of lower concentrations of bacteria was not tested in this study. In comparison, detection by ATP swab tests was reduced (Fig. 7). No difference was measured between the heat-killed and chlorine-stressed samples within each species, indicating that the presence of ATP in the bacterial cells was affected similarly for each species for both heat-killing and chlorine stress. The data demonstrate a 2-log reduction of detection for *E. coli* and *S. enterica* (Fig. 7) which could affect contaminant detection by ATP tests in the food industry. The detection of dead and stressed bacteria has important implications for the food industry, as they indicate sites of incomplete cleaning and previous sites of contamination, and organic surfaces upon which future contamination events could occur. Viable but nonculturable (VBNC) cells evade culture-based techniques to confirm the microbial safety of food (Highmore et al., 2018). Widespread use of chemical sanitizers such as bleach, chlorine, and peracetic acid can sublethally injure a subpopulation of bacterial contaminants and induce them into the VBNC state (Truchado et al., 2021), so detection at the macro-scale via Bactiscan may act as an effective prescreen for contamination following incomplete cleaning. Steam cleaning is an increasingly popular alternative to chemical sanitizers, where incomplete eradication of biofilm can provide ideal conditions for biofilm regrowth without the use of synergistic chemical treatments (Ban & Kang, 2016). The present study indicates that Bactiscan is capable of detecting heat-killed and sublethally injured bacterial contamination, but has a lower sensitivity than ATP test swabs in the detection of living cells. Used in isolation, Bactiscan is suitable for the detection of gross microbial contamination. We therefore propose that the two methods be used in conjunction, where Bactiscan can act as a macro-scale prescreen for areas of contamination which are then validated by ATP testing, thus negating the limited sample area of the swabs. Together, these methods are well placed to further improve cleaning practices in the food industry and mitigate the profound issue of VBNC evasion of routine microbial screening methods.

When applied to food contamination events comparable to that which could occur in the food industry, Bactiscan performed consistently despite the variation of different food types, surfaces, and species that comprised the bacterial contamination (Figs. 8–10). Some organic matter such as yoghurt fluoresced white (Fig. 9), however, bacterial contamination was observed under Bactiscan illumination as a bright turquoise color, contrasted against a blue background. The meat and fish samples produced less fluorescence on day one of the experiment when there was a lower microbial load (Figs. S2–S3) compared to samples after five days of incubation (Figs. 8–9), indicating that fluorescence of the sample correlates to microbial detection by Bactiscan. The high contrast between contamination and surface was maintained irrespective of surface material. Bactiscan was also able to illuminate thinner and smaller areas of contamination, particularly on the residues from the fish and meat. The budget competitor's UV torch produced fluorescence of inconsistent color in darkness (Fig. 9) and poor illumination in daylight (Fig. 8). It was heavily influenced by the reflection of the light from the surface, most prominently against steel, where the reflection from the surface masked any fluorescence from the surface (Figs. 8–9), and minimal fluorescence was observed at all on meat and fish on Teflon (Fig. 8). Overall, the budget UV torch was not as suitable for detection of microbial contamination on foods and surfaces.

Genome sequencing determined the primary sources of bacterial contamination of the tested foods, to the species level (Fig. 10). *Carnobacterium maltaromaticum* was present in all samples and was the most abundant species on each meat sample and one fish sample. *Carnobacterium maltaromaticum* is frequently isolated from meat and fish and can grow at low temperatures, but does not profoundly impact food spoilage (Casaburi et al., 2011; Leisner et al., 2007). *Corynebacterium* species have been isolated from foods, and some species have been used in food production (Tauch & Sandbote, 2014); however, *C. resistens* has been identified as a human pathogen with multidrug resistance (Otsuka et al., 2005; Schröder et al., 2012) and was present in the dairy sample incubated on polyurethane (Fig. 10). *S. aureus* was detected in seven of the nine food samples tested in this study, which is consistent with its prevalence on food samples, particularly in meat and dairy products (Bencardino et al., 2021; da Silva et al., 2020). The Bactiscan device illuminates microbial contamination, providing nonspecific detection of typical microflora in addition to foodborne pathogens and bacteria associated with food spoilage. Previous work has used metagenome sequencing to characterize the microbial communities of 109 food samples, and determined that pathogen reads contributed to 0.37% of the total (Bloomfield et al., 2023). The authors theorize that these low concentrations are misclassifications, but in the present study, the high concentration of *S. aureus* in the Fish Teflon sample (Fig. 10) supports the presence of the pathogen in other samples, as the fish samples shared an origin and the food samples were incubated close to one another. This work demonstrates that 'real world' contamination comprises mixed microbial communities where pathogens may be provided protection from cleaning procedures. This has been identified in biofilms of *L. monocytogenes* and *Lactiplantibacillus plantarum*, which have a higher tolerance to treatments of benzalkonium chloride and peracetic acid when grown together in biofilms (van der Veen & Abee, 2011). Due to these factors, the nonspecific detection nature of Bactiscan should not undermine its utility in identifying insufficiently clean areas in the food processing environment.

The results indicate that Bactiscan may be most effectively used by the industry in conjunction with ATP swab tests or other complementary technologies, integrating into the evaluation stage of factories' clean-in-place cycles. This complementary approach was also proposed in previous studies investigating hyperspectral detection methods (Beck et al., 2015), using the device as a screen for more targeted testing. However, the value of detection of dead and VBNC bacteria by Bactiscan permits the identification of past and future contamination events and helps to evaluate areas that require further sanitation treatments in accordance with current factory cleaning practices. Bactiscan could offer a simple-to-use, real-time response to detect biofilm, locating persistent sites of contamination to reduce the likelihood of foodborne disease outbreaks and improve infection prevention across the food industry.

Bactiscan is increasingly being used in the food industry to identify microbial contamination, but further work is necessary to refine its detection capability and reduce the variability in detection caused by human subjectivity. An important area of future research is to determine the molecular source of fluorescence and to investigate whether Bactiscan can distinguish between different bacterial species.

For example, the lower LOD50 values of *E. coli* and *S. enterica* relative to that of *L. monocytogenes* and *S. aureus* (Fig. 2) could be linked to their Gram-negative cell structure. This would require investigating a larger number of bacterial species and measuring the wavelengths of fluorescence under Bactiscan illumination to identify any differences between them. Furthermore, a limitation of this study was that planktonic bacteria were treated with heat and chemical sanitizers prior to Bactiscan detection but biofilms were not. To better understand the parameters of Bactiscan detection of real factory contamination, treated biofilms should be investigated in future work.

Finally, further investigation is required to reduce the impact of human error on the detection of microbial contamination. This study has determined that variation in human observation is significantly reduced when bacteria are illuminated by Bactiscan in darkness, although it should be noted that only three independent observers were used in this study which may not reflect the range of human observation ability across real-world users in the food industry. In the future, these findings could be applied to Bactiscan to produce an optimized, machine-assisted detection technique that integrates into clean-in-place practices throughout the food industry.

CRediT authorship contribution statement

Callum Highmore: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kirsty Cooper:** Writing – review & editing, Methodology. **Joe Parker:** Writing – review & editing, Methodology, Formal analysis. **Joshua Robinson:** Resources, Data curation. **Roberto Castangia:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Jeremy S. Webb:** Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 'Authors RC and JR are employed by EIT, which is the manufacturer of Bactiscan. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Roberto Castangia reports a relationship with EIT International that includes: employment. Joshua Robinson reports a relationship with EIT International that includes: employment. Authors RC and JR are employed by EIT International, which is the manufacturer of Bactiscan. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.'.

Acknowledgments

This work was funded by the Biotechnology and Biological Sciences Research Council and National Biofilms Innovation Centre FTMA3_21_027.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.jfp.2025.100511.

References

Amani, M. A., & Sarkodie, S. A. (2022). Mitigating spread of contamination in meat supply chain management using deep learning. *Scientific Reports, 12*(1), 5037. https://doi.org/10.1038/s41598-022-08993-5.

Bai, X., Nakatsu, C. H., & Bhunia, A. K. (2021). Bacterial biofilms and their implications in pathogenesis and food safety. *Foods, 10*(9), 2117.

Bai, X., Xu, L., Singh, A. K., Qiu, X., Liu, M., Abuzeid, A., ... Bhunia, A. K. (2022). Inactivation of polymicrobial biofilms of foodborne pathogens using epsilon poly-L-lysin conjugated chitosan nanoparticles. *Foods, 11*(4), 569.

Ban, G.-H., & Kang, D.-H. (2016). Effect of sanitizer combined with steam heating on the inactivation of foodborne pathogens in a biofilm on stainless steel. *Food Microbiology, 55*, 47–54. https://doi.org/10.1016/j.fm.2015.11.003.

Beck, E. A., Lefcourt, A. M., Lo, Y. M., & Kim, M. S. (2015). Use of a portable fluorescence imaging device to facilitate cleaning of deli slicers. *Food Control, 51*, 256–262. https://doi.org/10.1016/j.foodcont.2014.11.031.

Bencardino, D., Amagliani, G., & Brandi, G. (2021). Carriage of *Staphylococcus aureus* among food handlers: an ongoing challenge in public health. *Food Control, 130*, 108362. https://doi.org/10.1016/j.foodcont.2021.108362.

Bloomfield, S. J., Zomer, A. L., O'Grady, J., Kay, G. L., Wain, J., Janecko, N., ... Mather, A. E. (2023). Determination and quantification of microbial communities and antimicrobial resistance on food through host DNA-depleted metagenomics. *Food Microbiology, 110*, 104162. https://doi.org/10.1016/j.fm.2022.104162.

Bottari, B., Santarelli, M., & Neviani, E. (2015). Determination of microbial load for different beverages and foodstuff by assessment of intracellular ATP. *Trends in Food Science & Technology, 44*(1), 36–48. https://doi.org/10.1016/j.tifs.2015.02.012.

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: a review. *Biofouling, 27*(9), 1017–1032. https://doi.org/10.1080/08927014.2011.626899.

Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., Piard, J. C., Naïtali, M., & Briandet, R. (2015). Biofilm-associated persistence of food-borne pathogens. *Food Microbiology, 45*, 167–178. https://doi.org/10.1016/j.fm.2014.04.015.

Casaburi, A., Nasi, A., Ferrocino, I., Di Monaco, R., Mauriello, G., Villani, F., & Ercolini, D. (2011). Spoilage-related activity of *Carnobacterium maltaromaticum* strains in air-stored and vacuum-packed meat. *Applied and Environmental Microbiology, 77*(20), 7382–7393. https://doi.org/10.1128/aem.05304-11.

da Silva, A. C., Rodrigues, M. X., & Silva, N. C. C. (2020). Methicillin-resistant *Staphylococcus aureus* in food and the prevalence in Brazil: a review. *Brazilian Journal of Microbiology, 51*(1), 347–356. https://doi.org/10.1007/s42770-019-00168-1.

Daniel, N., Casadevall, N., Sun, P., Sugden, D., & Aldin, V. (2020). *The Burden of Foodborne Disease in the UK 2018*. London: FSA.

Fagan, R. P., & Fairweather, N. F. (2014). Biogenesis and functions of bacterial S-layers. *Nature Reviews Microbiology, 12*(3), 211–222. https://doi.org/10.1038/nrmicro3213.

Fetsch, A., & Johler, S. (2018). *Staphylococcus aureus* as a foodborne pathogen. *Current Clinical Microbiology Reports, 5*(2), 88–96. https://doi.org/10.1007/s40588-018-0094-x.

Foddai, A. C. G., & Grant, I. R. (2020). Methods for detection of viable foodborne pathogens: current state-of-art and future prospects. *Applied Microbiology and Biotechnology, 104*(10), 4281–4288. https://doi.org/10.1007/s00253-020-10542-x.

Gowen, A. A., Feng, Y., Gaston, E., & Valdramidis, V. (2015). Recent applications of hyperspectral imaging in microbiology. *Talanta, 137*, 43–54. https://doi.org/10.1016/j.talanta.2015.01.012.

Gray, M. J., Wholey, W.-Y., & Jakob, U. (2013). Bacterial responses to reactive chlorine species. *Annual Review of Microbiology, 67*, 141–160. https://doi.org/10.1146/annurev-micro-102912-142520.

Habimana, O., Meyrand, M., Meylheuc, T., Kulakauskas, S., & Briandet, R. (2009). Genetic features of resident biofilms determine attachment of *Listeria monocytogenes*. *Applied and Environmental Microbiology, 75*(24), 7814–7821. https://doi.org/10.1128/aem.01333-09.

Hammons, S. R., Stasiewicz, M. J., Roof, S., & Oliver, H. F. (2015). Aerobic plate counts and ATP levels correlate with *Listeria monocytogenes* detection in retail delis. *The Journal of FoodProt, 78*(4), 825–830. https://doi.org/10.4315/0362-028x.Jfp-14-500.

Han, L., Wang, K., Ma, L., Delaquis, P., Bach, S., Feng, J., & Lu, X. (2020). Viable but nonculturable *Escherichia coli* O157:H7 and *Salmonella enterica* in fresh produce: rapid determination by loop-mediated isothermal amplification coupled with a propidium monoazide treatment. *Applied and Environmental Microbiology, 86*(7). https://doi.org/10.1128/aem.02566-19.

He, H.-J., & Sun, D.-W. (2015). Hyperspectral imaging technology for rapid detection of various microbial contaminants in agricultural and food products. *Trends in Food Science & Technology, 46*(1), 99–109. https://doi.org/10.1016/j.tifs.2015.08.001.

Highmore, C. J., Warner, J. C., Rothwell, S. D., Wilks, S. A., & Keevil, C. W. (2018). Viable-but-nonculturable *Listeria monocytogenes* and *Salmonella enterica* serovar thompson induced by chlorine stress remain infectious. *MBio, 9*(2)e00540-00518. https://doi.org/10.1128/mBio.00540-18.

Jun, W., Kim, M. S., Cho, B.-K., Millner, P. D., Chao, K., & Chan, D. E. (2010). Microbial biofilm detection on food contact surfaces by macro-scale fluorescence imaging. *Journal of Food Engineering, 99*(3), 314–322. https://doi.org/10.1016/j.jfoodeng.2010.03.005.

Leisner, J. J., Laursen, B. G., Prévost, H., Drider, D., & Dalgaard, P. (2007). Carnobacterium: positive and negative effects in the environment and in foods. *FEMS Microbiology Reviews, 31*(5), 592–613. https://doi.org/10.1111/j.1574-6976.2007.00080.x.

Li, B., & Chen, J.-Q. (2012). Real-time PCR methodology for selective detection of viable *Escherichia coli* O157:H7 cells by targeting Z3276 as a genetic marker. *Applied and Environmental Microbiology, 78*(15), 5297–5304. https://doi.org/10.1128/AEM.00794-12.

Li, B., & Chen, J.-Q. (2013). Development of a sensitive and specific qPCR assay in conjunction with propidium monoazide for enhanced detection of live *Salmonella*

spp. in food. *BMC Microbiology, 13*(1), 273. https://doi.org/10.1186/1471-2180-13-273.

Luo, J., Liu, X., Tian, Q., Yue, W., Zeng, J., Chen, G., & Cai, X. (2009). Disposable bioluminescence-based biosensor for detection of bacterial count in food. *Analytical Biochemistry, 394*(1), 1–6. https://doi.org/10.1016/j.ab.2009.05.021.

Manville, E., Kaya, E. C., Yucel, U., Boyle, D., & Trinetta, V. (2023). Evaluation of *Listeria monocytogenes* biofilms attachment and formation on different surfaces using a CDC biofilm reactor. *International Journal of Food Microbiology, 399*, 110251. https://doi.org/10.1016/j.ijfoodmicro.2023.110251.

Martinović, A., Cocuzzi, R., Arioli, S., & Mora, D. (2020). *Streptococcus thermophilus*: to survive, or not to survive the gastrointestinal tract, that is the question! *Nutrients, 12*(8). https://doi.org/10.3390/nu12082175.

Merda, D., Felten, A., Vingadassalon, N., Denayer, S., Titouche, Y., Decastelli, L., ... Hennekinne, J.-A. (2020). NAuRA: genomic tool to identify *Staphylococcal Enterotoxins* in *Staphylococcus aureus* strains responsible for FoodBorne outbreaks. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01483.

Otsuka, Y., Kawamura, Y., Koyama, T., Iihara, H., Ohkusu, K., & Ezaki, T. (2005). *Corynebacterium resistens* sp. nov., a New Multidrug-Resistant Coryneform Bacterium Isolated from Human Infections. *J Clin Microbiol, 43*(8), 3713–3717. https://doi.org/10.1128/jcm.43.8.3713-3717.2005.

Pires, S. M., & Devleesschauwer, B. (2021). Chapter 1 - Estimates of global disease burden associated with foodborne pathogens. In J. G. Morris & D. J. Vugia (Eds.), *Foodborne Infections and Intoxications* (Fifth Edition, pp. 3–17). Academic Press.

Prouty, A. M., Schwesinger, W. H., & Gunn, J. S. (2002). Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. *Infect Immun, 70*(5), 2640–2649. https://doi.org/10.1128/iai.70.5.2640-2649.2002.

Robben, C., Witte, A. K., Schoder, D., Stessl, B., Rossmanith, P., & Mester, P. (2019). A fast and easy ATP-based approach enables MIC testing for non-resuscitating VBNC pathogens. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.01365.

Russell, A. (2003). Lethal effects of heat on bacterial physiology and structure. *Science Progress, 86*(1–2), 115–137.

Saadi, M., Haddar, A., Ferjani, H., Troudi, M., & Ncib, M. (1998). La surveillance des toxi-infections alimentaires collectives dans la région de Sousse: Rétrospective de neuf ans. *Tunisie Médicale, 76*(12), 448–454.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... Griffin, P. M. (2011). Foodborne illness acquired in the United States–major pathogens. *Emerging Infectious Diseases, 17*(1), 7–15. https://doi.org/10.3201/eid1701.p11101.

Schmid-Hempel, P., & Frank, S. A. (2007). Pathogenesis, virulence, and infective dose. *PLoS Pathogens, 3*(10), 1372–1373. https://doi.org/10.1371/journal.ppat.0030147.

Schröder, J., Maus, I., Meyer, K., Wördemann, S., Blom, J., Jaenicke, S., ... Tauch, A. (2012). Complete genome sequence, lifestyle, and multi-drug resistance of the human pathogen *Corynebacterium resistens* DSM 45100 isolated from blood samples of a leukemia patient. *BMC Genomics, 13*, 141. https://doi.org/10.1186/1471-2164-13-141.

Simões, M., Pereira, M. O., & Vieira, M. J. (2003). Effect of different concentrations of ortho-phthalaldehyde on biofilms formed by *Pseudomonas fluorescens* under different flow conditions. *Biofouling, 19*(5), 287–295. https://doi.org/10.1080/0892701031000153398.

Soni, A., Dixit, Y., Reis, M. M., & Brightwell, G. (2022). Hyperspectral imaging and machine learning in food microbiology: developments and challenges in detection of bacterial, fungal, and viral contaminants. *Comprehensive Reviews in Food Science and Food Safety, 21*(4), 3717–3745. https://doi.org/10.1111/1541-4337.12983.

Tauch, A., & Sandbote, J. (2014). The family corynebacteriaceae. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The prokaryotes: actinobacteria* (pp. 239–277). Berlin, Heidelberg: Springer Berlin Heidelberg.

Truchado, P., Gil, M. I., & Allende, A. (2021). Peroxyacetic acid and chlorine dioxide unlike chlorine induce viable but non-culturable (VBNC) stage of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in wash water. *Food Microbiology, 100*, 103866. https://doi.org/10.1016/j.fm.2021.103866.

van der Veen, S., & Abee, T. (2011). Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. *International Journal of Food Microbiology, 144*(3), 421–431. https://doi.org/10.1016/j.ijfoodmicro.2010.10.029.

Van Meervenne, E., De Weirdt, R., Van Coillie, E., Devlieghere, F., Herman, L., & Boon, N. (2014). Biofilm models for the food industry: hot spots for plasmid transfer? *Pathogens and Disease, 70*(3), 332–338. https://doi.org/10.1111/2049-632x.12134.

Wang, R., King, D. A., & Kalchayanand, N. (2022). Evaluation of *Salmonella* biofilm cell transfer from common food contact surfaces to beef products. *Journal of Food Protection, 85*(4), 632–638. https://doi.org/10.4315/JFP-21-334.

WHO. (2015). *WHO estimates of the global burden of foodborne diseases*.

Wiederoder, M. S., Liu, N., Lefcourt, A. M., Kim, M. S., & Martin Lo, Y. (2013). Use of a portable hyperspectral imaging system for monitoring the efficacy of sanitation procedures in produce processing plants. *Journal of Food Engineering, 117*(2), 217–226. https://doi.org/10.1016/j.jfoodeng.2013.02.019.

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. *Genome Biology, 20*(1), 257. https://doi.org/10.1186/s13059-019-1891-0.

Zeng, D., Chen, Z., Jiang, Y., Xue, F., & Li, B. (2016). Advances and challenges in viability detection of foodborne pathogens. *Frontiers in Microbiology, 7*. https://doi.org/10.3389/fmicb.2016.01833.